Studies on the Internal Defense Mechanisms of Sponges

I. The Cell Types Occurring in the Mesoglea of *Terpios zeteki* (de Laubenfels) (Porifera: Demospongiae)[1]

THOMAS C. CHENG, HERBERT W. F. YEE, and ERIK RIFKIN[2]

IT IS WELL KNOWN that various morphological types of free cells occur in the mesoglea of sponges. Collectively, these cells are generally designated as amoebocytes since they are all assumed to be capable of amoeboid movement although their functions may vary. According to Hyman (1940), in those poriferan species in which there are much mesoglea and relatively few cells, the mesenchyme, that is, the mesoglea together with the cells embedded therein, may be referred to as the collenchyma, while in those in which there are numerous cells, the mesenchyme may be designated as the parenchyma. The mesenchyme of the species under consideration may be considered parenchymatous. A search of the literature pertaining to the mesenchymal cells of sponges reveals that a variety of names have been coined to designate the various cell types (Tuzet, 1932; de Laubenfels, 1932; Wilson and Penney, 1930; and others). This practice has led to considerable confusion for those interested in cell homologies and analogies. During our investigation of the parenchymal cells of *Terpios zeteki* (de Laubenfels), we have found the designations used by Minchin (1900), Galtsoff (1925), and Hyman (1940) categorizing the free cells as archaeocytes, collencytes, chromocytes, thesocytes, and scleroblasts, to be the most useful. Therefore, we have used these names for the cells encountered in *T. zeteki.*

Our interest in poriferan collenchymal or parenchymal cells stems from inquiries into the types of internal defense mechanisms occurring in sponges, whether in response to abiotic foreign particles or to microsymbionts, including parasites. Although studies of this nature, particularly the various aspects of cellular defense mechanisms (leucocytosis, phagocytosis, pinocytosis, and encapsulation) have been examined extensively among the higher coelomate invertebrates, especially the Mollusca and Arthropoda, very few comparable studies have been carried out among the Porifera. In this paper are described the various types of free amoebocytes normally found in the parenchyma of *T. zeteki,* and the ratio of each type of cell present is reported.

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MATERIALS AND METHODS

Terpios zeteki is one of the two or three most abundant species of sponges in Hawaii (de Laubenfels, 1950). Specimens collected from pilings at Ala Wai Yacht Harbor, Honolulu, were brought into the laboratory and maintained in aerated filtered sea water with a salinity of 35 ‰ at 20°C. The parenchymal cells were examined by two methods: in histological sections, and in smears of living tissues.

Histological sections of *T. zeteki* were prepared by fixing segments of whole sponges, each approximately 10 cm in length, for 12 hours in 10% neutral formalin. These were subsequently dehydrated via a closely graded ethanol series, cleared in xylene, and embedded in high temperature paraffin (melting point 56°C). The sections were cut at 10μ and stained with Harris' hematoxylin and counterstained with eosin. After the morphological characteristics of each type of cell found in the mesoglea were determined, comparable cells were sought in smear preparations of small pieces of living sponges, each piece measuring approximately 0.5 cm^3. Examinations of living dissociated

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[2] Laboratory of Symbiology, Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822.

parenchymal cells were made as these revealed certain distinguishing characteristics not readily observable in stained sections. For example, the pseudopodial movements of each type of cell and their cytoplasmic inclusions, especially the pigments of chromocytes, were clearly visible only in living cells. The dimensions of the cells were determined from living cells by use of a calibrated ocular micrometer.

The percentages of the total number of parenchymal cells represented by each cell type were determined in the following manner. Uniform suspensions of dissociated cells of 10 sponges were made in filtered sea water. Samples of such suspensions were examined with a phase-contrast microscope equipped with a Whipple-Hausser ocular micrometer to facilitate counting. During the counting procedure, the first hundred cells recognized as amoebocytes native to the mesoglea encountered in the squares were recorded by type. A total of 23 counts were made.

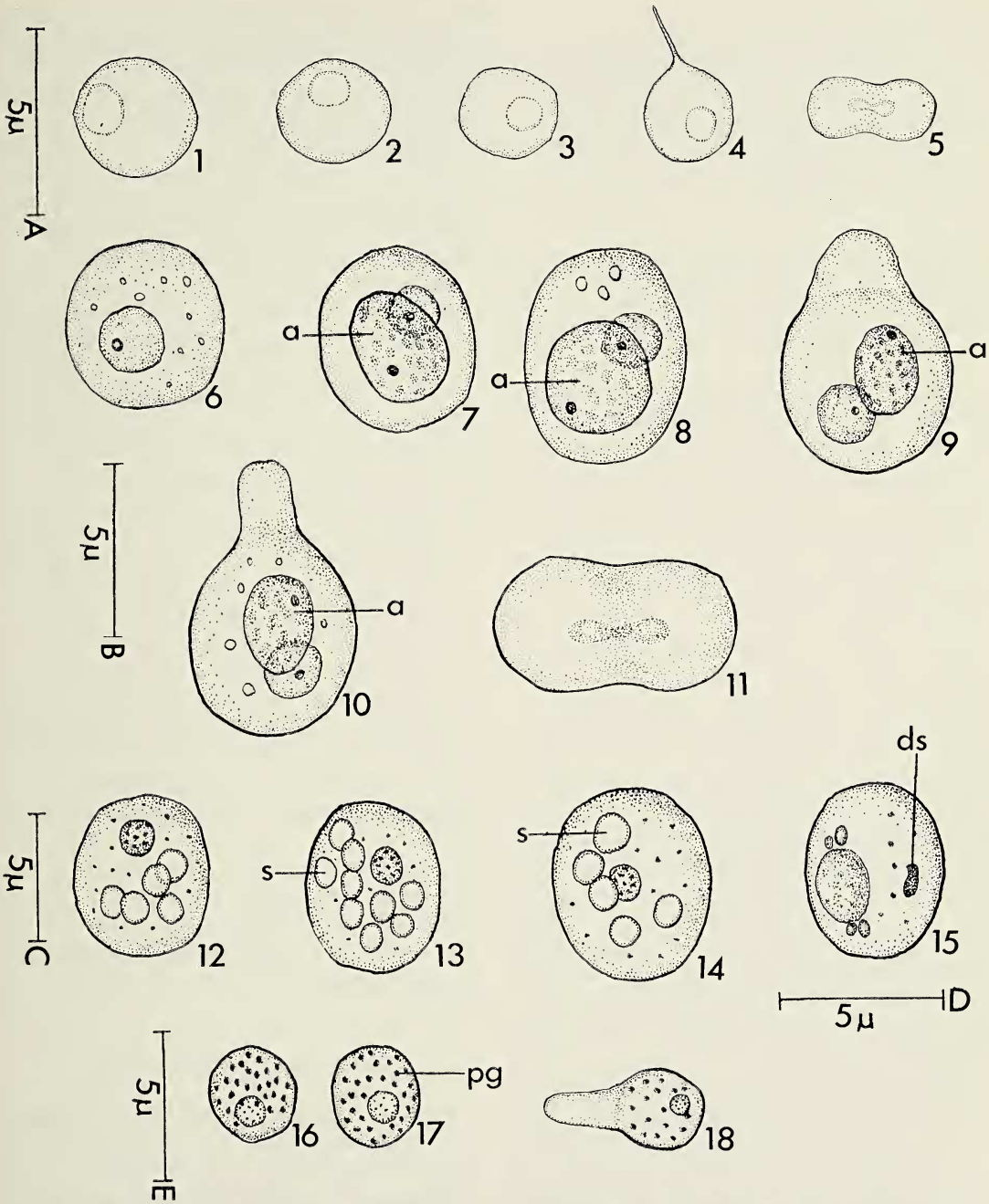
RESULTS

Descriptions of Cell Types

The architecture of *Terpios zeteki* is of the leuconoid type. The viscous mesogleal layer fills the spaces between the flagellated chambers and canals and is tightly packed with cells and spicules. The most abundant type of cell is the small spherical *collencyte,* each measuring 0.003 mm (0.002–0.004 mm) in diameter (Figs. 1–5). When examined in histological sections, many of the collencytes possess fine pseudopodia which are fused with those of adjacent cells to form a netlike syncytium (Fig. 19) with independent cells intermingled with it. In preparations of dissociated cells, however, collencytes generally round up, appearing as minute spheres which are not interconnected. Occasionally one is seen producing one or more fine pseudopodia (Fig. 4). In hematoxylin-and-eosin stained sections, the cytoplasm of collencytes is agranular and is either chromophobic or only slightly eosinophilic while the nucleus is homogeneously slightly hematoxyphilic. These cells are limited to the parenchyma although occasionally collencytes have been observed free in the water canals, particularly excurrent canals. When dissociated living collencytes are observed under the light microscope, the cytoplasm appears clear but the nucleus is extremely difficult to define. When examined with phase-contrast microscopy, however, each nucleus appears to be homogeneous and rounded. On a few occasions the nuclei of collencytes have been observed dividing although the exact mitotic figures have not been studied (Fig. 5).

The second most abundant type of parenchymal cell is the rounded to ovoid *archaeocyte* which measures 0.006 by 0.005 mm (0.004–0.007 by 0.004–0.007 mm) (Figs. 6–11). This type of cell appears either rounded or with a lobose pseudopodial projection in histological sections. Many of them enclose a rather large globose to ovoid hematoxyphilic body that measures 0.003 mm in greatest diameter and which when observed in the living state proved to be a yellowish-green symbiotic zooxanthella with a distinct bright red stigma. In addition to the zooxanthella, each archaeocyte includes a rounded nucleus with a distinct nucleolus, and some include cytoplasmic inclusions which vary in size, while others include one or two vacuoles. The cytoplasm of these cells is faintly eosinophilic while the nucleus is hematoxyphilic. It should be noted that very rarely the pseudopods of two adjacent archaeocytes may be fused to form a two-celled syncytium. In addition, archaeocytes are occasionally found free in the water canals, primarily the excurrent canals. When examined in the living state, archaeocytes are either spherical or ovoid. Approximately 45–50% of those encountered include endosymbiotic zooxanthellae. The cytoplasm may be clear or with a few hyaline inclusions. These cells have been observed to produce a single lobopodium (Figs. 9, 10). Occasionally some have been observed undergoing what appears to be division (Fig. 11); this, however, is not a common phenomenon. Approximately one out of 60 cells is dividing.

The third type of parenchymal cell encountered in *T. zeteki* is believed to represent *thesocytes.* Each of these measures 0.007 by 0.006 mm (0.005–0.010 by 0.004–0.009 mm). According to Hyman (1940), thesocytes are nutrient-enclosing cells. The exact chemical nature of the cytoplasmic inclusions of what we are designating as thesocytes has not been determined although the consistent occurrence of these



Figs. 1–18. Cell types found in the parenchyma of *Terpios zeteki*. Drawn from living material.

1–5. Collencytes. Notice the fine filament-like pseudopod in *4* and the dividing collencyte in *5*. (Scale A)

6–11. Archaeocytes. Notice the presence of a symbiotic zooxanthella (*a*) in *7–10*, the formation of pseudopods in *9* and *10*, and a dividing cell in *11*. (Scale B)

12–14. Thesocytes. Notice two types of cytoplasmic inclusions, the larger bodies (*s*), and extremely small granules. (Scale C)

15. Scleroblast. Notice the intracytoplasmically developing spicule (*ds*). (Scale D)

16–18. Chromocytes. Notice the pigment granules (*pg*) in all the cells depicted and the formation of a pseudopod in *18*. (Scale E)

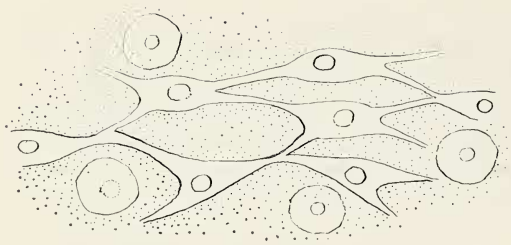


FIG. 19. Syncytially arranged collencytes embedded in the mesoglea of *Terpios zeteki*. Drawn from section stained with hematoxylin and eosin. Notice unconnected collencytes intermingled with syncytially arranged ones.

inclusions, which resemble stored nutrient globules, suggests their functional nature. There are two types of cytoplasmic inclusions. The more predominant is in the form of relatively large globose bodies each measuring approximately 0.002 mm in diameter. The second type is in the form of small granules which are barely visible under the light microscope. The larger globules in living cells do not give a positive test for glycogen when stained with Lugol's iodine but, when stained with Sudan Black B, only some give a positive test for lipids. In H and E stained sections, the cytoplasm of thesocytes is eosinophilic. Some of the larger inclusion bodies are also eosinophilic, others appear yellowish, while still others are chromophobic. Among the smaller granules, some are eosinophilic while others are colorless. The nuclei, each measuring approximately 0.002 mm in diameter, are strongly hematoxyphilic with distinct chromatin granules but no visible nucleoli. When observed in the living state, the larger inclusions vary from yellowish to colorless, while the smaller granules range from colorless to dark brown.

The fourth type of parenchymal cell includes the *chromocytes* or pigment-bearing cells (Figs. 16–18). Actually two types of chromocytes, based on the color of the pigments present, are distinguishable: those enclosing red, and those with black, pigment granules. Both types of cells are of the same size, measuring 0.004 by 0.003 mm (0.002–0.005 by 0.003–0.005 mm). These cells are distributed primarily in those regions of the mesoglea situated near the body surface of the sponge. Undoubtedly it is the red pigment-bearing chromocytes that give *T. zeteki* its reddish surface color and the black pigmented ones that give certain areas their blackish color. Each cell includes a nucleus, measuring 0.0008 mm in diameter, with distinct chromatin granules but no visible nucleolus. The pigment granules are more or less evenly distributed through the cytoplasm. In H and E stained sections, these pigment granules are deeply hematoxyphilic. Living chromocytes are capable of amoeboid movement (Fig. 18).

Skeleton-secreting cells or *scleroblasts* are rarely encountered in *T. zeteki*. Examination of sections of 10 sponges revealed only two, while in the numerous smear preparations examined, only nine scleroblasts, all silicoblasts (silicious spicule-forming cells), were observed. These cells, measuring 0.005 mm in greatest diameter, are quite similar to archaeocytes except for their slightly larger nuclei, which are devoid of nucleoli, and the presence of a developing spicule in each (Fig. 15). The young spicule is noncellular, colorless, and refractile. Its shape is generally elongate, with knobs at one or both terminals. In addition to the nucleus and young spicules, two types of cytoplasmic inclusions occur. The first is in the form of ovoid hematoxyphilic globules which are generally located near the nucleus. The second is represented by extremely minute colorless granules randomly distributed in the cytoplasm (Fig. 15).

Cell Counts and Ratios

A total of 23 differential counts were made of the cell types in *T. zeteki* by the method described earlier. The data are tabulated in Table 1.

DISCUSSION AND CONCLUSIONS

From our examination of both histological sections and dissociated cells it is evident that the parenchymal cells of *Terpios zeteki* can be identified as belonging to five cell types: collencytes, archaeocytes, thesocytes, chromocytes, and scleroblasts. These can be distinguished by their dimensions, cytoplasmic inclusions, and other characteristics. It is of interest to note that collencytes, which represent the most abundant type of cell, are capable of forming syncytia by the fusion of their fine pseudopodia. A similar

TABLE 1

Results of 23 Differential Counts of the Five Types of Free Cells Found in the Parenchyma of *Terpios zeteki*

COUNT NO.	COLLENCYTES	ARCHAEOCYTES	CHROMOCYTES	THESOCYTES	SCLEROBLASTS
1	68	14	12	5	1
2	64	10	18	8	0
3	68	14	8	10	0
4	52	34	8	4	2
5	58	20	14	6	2
6	62	18	12	8	0
7	64	20	6	8	2
8	58	22	12	8	0
9	56	20	10	14	0
10	58	24	10	8	0
11	54	26	16	4	0
12	58	24	14	4	0
13	54	30	4	12	0
14	52	20	22	6	0
15	56	24	14	6	0
16	52	24	22	2	0
17	52	24	18	6	0
18	52	32	10	4	2
19	50	24	8	18	0
20	58	24	14	4	0
21	66	24	6	4	0
22	58	22	12	8	0
23	60	18	16	6	0
Total	1330	512	286	163	9
Mean ± S.D.	57.83 ± 5.39	22.26 ± 5.54	12.43 ± 4.82	7.09 ± 3.68	0.39 ± 2.2

phenomenon has been reported by Tuzet (1932) who described what she termed "stellate cells" in *Regiera elegans* (Bwk.) and *R. simulans* (Johnston) with anastomosing pseudopods. It is uncertain whether Tuzet's "stellate cells" are homologous with *T. zeteki* collencytes, as she reported the presence in the former of a granular nucleus containing a nucleolus. As we have noted, nuclei of *T. zeteki* collencytes are agranular and without visible nucleoli. Similarly, Wilson and Penney (1930), who examined *Microciona prolifera* Verrill, and de Laubenfels (1932), who studied *Iotrochota birotulata* Higgin, have reported that many of the mesenchymal (parenchymal) cells in these sponges are connected by fine protoplasmic processes. Specifically, Wilson and Penney stated: "The space not occupied by canals, flagellated chambers, and skeletal fibers is filled with a tissue here designated as mesenchyme. It consists of abundant cells of several kinds, very many of which are connected together by intercellular strands. . . ." This situation, although superficially similar, is definitely different from that found in *T. zeteki.* In this sponge, collencytes are connected only with other collencytes and have not been observed to fuse with any other type of cell. Van Weel (1949), in a study of the freshwater sponge *Spongilla proliferens* Annand, has also reported syncytially arranged "indifferent cells" with faintly stained clear cytoplasm, and which may be without a nucleolus. His "indifferent cells" are believed to be comparable to our collencytes. It is proposed that the collencytes of *T. zeteki* represent the basic type of structural cell *in situ* and provide a firm network which, together with the spongin fibers and spicules, add stability to the integrity of the parenchyma. The "stellate cells" of *Regiera* spp., as described by Tuzet (1932), and the "indifferent cells" of *S. proliferens,* as described by van Weel (1949), appear to serve the same function. It is noted, however, that not all of the collencytes of *T. zeteki* are fused. Independent collencytes also occur. It is also of interest to note that the

intercellular connections between collencytes are extremely fragile and are readily broken. Such connections are not found in smear preparations.

As stated, archaeocytes, which also occur in relatively large numbers in *T. zeteki,* are often found to include zooxanthellae. It is of interest to note that according to McLaughlin and Zahl (1966), the terms "zoochlorellae" and "zooxanthellae" are not generic designations but their contemporary meanings are primarily coloristic. Zooxanthellae are yellowish to greenish-brown algal cells, while zoochlorellae are pale to bright green cells. Those found in *T. zeteki* archaeocytes are yellowish-green and therefore are designated as zooxanthellae.

The occurrence of symbiotic algae, zooxanthellae or zoochlorellae, in sponges has been known since the investigations of Weber and Weber-van Bosse (1890), Koorders (1902), van Trigt (1919), Rodriguez (1930), van Weel (1949), and others. The sponge cells enclosing such algal cells have been designated by a variety of names. As examples, van Weel (1949) refers to the cells of *Spongilla proliferens* as "amoebocytes with symbiontic algae" (abbreviated "ASA") and infers that they are different from "phagocytes," and Rodriguez (1930) refers to such cells in *Spongilla lacustris* Johnston as "amoebocytes." An examination of the descriptions of these host cells has convinced us that such cells should all be designated as archaeocytes in that they all are relatively large, are capable of producing lobose pseudopodia, possess prominent nuclei with nucleoli, and may include cytoplasmic granules and vacuoles. In fact, Pourbaix (1933), in reviewing Rodriguez' work, has referred to these cells as archaeocytes.

It may be significant that symbiotic algal cells are found only in archaeocytes and not in other types of cells. Our studies on the phagocytic roles of the various types of parenchymal cells in *T. zeteki,* which will be published at a later date, indicate that archaeocytes are much more active and efficient in phagocytizing foreign materials. According to Rodriguez, the algal cells enter sponges via the flagellated choanocytes and are later transferred to parenchymal amoebocytes (=archaeocytes). As the result of finding algal cells in the process of being digested within archaeocytes, van Trigt (1919) concluded that the relationship between the alga and the sponge is not a completely compatible one; however, van Weel (1949) has shown that sponges, in his case *Spongilla proliferens,* are associated with only one species of alga, in his case *Pleurococcus vulgaris,* thus suggesting specificity, and having found few examples of intracellular digestion of *P. vulgaris,* he disagrees with van Trigt that the relationship is not totally compatible. Van Weel is of the opinion that only dying or dead algae become digested. Our examination of zooxanthellae within *T. zeteki* archaeocytes revealed very few instances of intracellular digestion and we tend to agree with van Weel that the relationship is one of compatible symbiosis, most probably mutualism as defined by Cheng (1967).

The nature of the cytoplasmic inclusions of *T. zeteki* thesocytes remains essentially unknown. Our preliminary studies indicate that the larger globules do not represent glycogen. On the other hand, certain ones give a positive test for lipids. It is of interest to note that Pourbaix (1934) has reported that the nutrient reserves in certain cells comprising the gemmules of *Ephydatia fluviatilis* Lamouroux are not glycogen but may be proteinaceous, probably a glycoprotein. In addition, he has found unidentified lipoid globules. Similarly, van Weel (1949) reported that no glycogen occurs in the cells of adult *Spongilla proliferens* except in ovocytes and that fats occur in what he termed "phagocytes." Our findings confirm the presence of lipids and the absence of glycogen in the nutrient-storing cells of sponges. Furthermore, it is possible that those globules which do not give a positive stain with either Sudan Black B or Lugol's iodine may represent glycoprotein granules. This, however, is pure speculation and needs histochemical confirmation.

Our finding of intracellular formation of spicules in the few scleroblasts encountered indicates that in *T. zeteki* the spicules are formed intracellularly, at least initially, although the exact machanisms involved remain to be determined.

As we have stated, our interest in the parenchymal cells of sponges stems from inquiries into their roles as associated with cellular internal defense mechanisms. With the estab-

lishment of the types of cells in *T. zeteki,* it is now possible to examine the phagocytic role of each type. Furthermore, with the establishment of the normal ratios of each type of cell, we now have the baseline for determining whether increases of all or certain types of amoebocytes occur when challenged with foreign materials. These studies are currently in progress.

SUMMARY

The parenchymal cells of the sponge *Terpios zeteki* were studied both in histological sections and in smear preparations. Five distinct morphological types can be recognized. These are readily identified as collencytes, archaeocytes, thesocytes, chromocytes, and scleroblasts. The dimensions and morphological characteristics of each type are given. An endocytoplasmic symbiotic zooxanthella occurs in the archaeocytes of *T. zeteki.* Counts revealed that collencytes are the most abundant of the five cell types. Many of these are syncytially arranged *in situ.* Archaeocytes are the next most abundant, followed by chromocytes, thesocytes, and scleroblasts. Intracytoplasmic spicule formation was observed in scleroblasts. Having defined the cell types and ratios occurring in the mesoglea of *T. zeteki,* it is now possible to examine the role of each as associated with phagocytosis and other forms of internal cellular defense mechanisms.

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